MICROBAC®

Microbac Protocol

Testing Pre-Saturated or Impregnated Towelettes for Hard Surface Disinfection

Staphylococcus aureus

Testing Facility
Microbac Laboratories, Inc.
105 Carpenter Drive
Sterling, VA 20164

Prepared for
Professional Disposables International, Inc.
Two Nicepak Park
Orangeburg, NY 10962

October 12, 2018

Microbac Protocol: 735.1.10.12.18
Microbac Project No.: 735 - 316

OBJECTIVE:

This test is designed to substantiate disinfectant effectiveness for impregnated or pre-saturated towelettes, single or multiple uses, to be registered with the Environmental Protection Agency and Health Canada. The test evaluates the effectiveness of products as disinfectants for contaminated surfaces. The test follows the "Germicidal Spray Products as Disinfectants" test as described in the Official Methods of Analysis, Eighteenth Edition, 2012, AOAC and the EPA Notice of Efficacy Requirements for Pre-Saturated or Impregnated Towelettes for Hard Surface Disinfection. This test also meets the EPA OCSPP 810.2000 and 810.2200 Product Performance Test Guidelines and Health Canada "Guidance Document – Safety and Efficacy Requirements for Hard Surface Disinfectant Drugs" as applicable.

TESTING CONDITIONS:

Using a single test substance (1), per lot (3), sixty carriers (60), per microorganism (1), per contact time (1) will be evaluated (total of 180 replicates). Carriers inoculated with *Staphylococcus aureus* will be wiped as directed by the sponsor or label instruction and held for the exposure time and at the temperature specified by the sponsor. The carriers will be cultured, incubated and observed for visible growth.

MATERIALS:

A. Test, control and reference substances will be supplied by the sponsor of the study (see last page). As per CFR 40.160.105:

- The identity, strength, purity, and composition, or other characteristics which will appropriately define the test, control, or reference substance shall be determined for each lot and shall be documented by the sponsor before its use in a study. Methods of synthesis, fabrication, or derivation of the test, control, or reference substance shall be documented and retained by the sponsor.

- When relevant to the conduct of the study the solubility of each test, control, or reference substance shall be determined by the sponsor before the experimental start date. The stability of the test, control, or reference substance shall be determined by the sponsor before the experimental start date or concomitantly according to written standard operating procedures, which provide for periodic analysis of each lot.

The test substance will be tested as supplied by the sponsor unless directed otherwise. All operations performed on the test substance such as dilution or specialized storage conditions must be specified by the sponsor before initiation of testing.

The sponsor assures Microbac Laboratories, Inc. (Microbac) testing facility management that the test substance has been appropriately tested for identity, strength, purity, stability, and uniformity as applicable.

Microbac will retain all unused test substances for a period of at least one year after completion of the test, and then discard them in a manner that meets the approval of the safety officer.

B. Materials supplied by Microbac, including, but not limited to:

1. Challenge microorganisms, required by the sponsor of the study: *Staphylococcus aureus*, ATCC 6538

2. Media and reagents:
   a. Synthetic Broth (SB)
   b. Neutralizer: Recovery broth with required neutralizer(s)
   c. Letheen Broth (LB)
   d. Heat-inactivated Fetal Bovine Serum (if required)
   e. Phosphate Buffered Dilution Water (PBDW)
   f. Tryptic Soy Agar (TSA)
   g. Mannitol Salt Agar (MSA)

3. Laboratory equipment and supplies, including glass microscope slides (1" x 3" with a 1" x 1" surface for contamination and treatment)

TEST SYSTEM IDENTIFICATION:

As applicable, all test and control tube racks will be labeled with microorganism, test substance, lot identifier, and project number prior to initiation of the study and during incubation. Petri dishes will be labeled with microorganism prior to initiation of the study and microorganism and project number during incubation. Test substance and usage will be traced according to SOPs extant in the laboratory.

EXPERIMENTAL DESIGN:

A. Inoculum preparation:

A single frozen cryovial of stock culture will be defrosted at room temperature and then briefly vortexed to mix. A 10 µL aliquot of the thawed stock will be added to a tube containing 10 mL of SB.

The tubes will be vortexed and incubated at 36±1C for 24±2 hours. Daily transfers will be made for at least one but no more than five consecutive days.

For the final subculture transfer, tubes containing 10 mL SB will be inoculated with 10 µL of culture per tube and incubated at 36±1C. After 48-54 hours, cultures will be used for contaminating the carriers.

The cultures will be agitated on a Vortex-type mixer for 3-4 seconds, and then allowed to sit for 10 minutes.

The upper portion of each culture will be removed, leaving any debris or clumps and transferred to a sterile flask and pooled.

If requested by the sponsor, heat-inactivated fetal bovine serum will be added to the cultures to achieve an organic load of 5%.

B. Carrier preparation and inoculation:

The new carriers will be visually screened and discarded if visibly damaged (scratched, chipped or nicked). The carriers will be rinsed with 95% ethanol followed by a rinse with deionized water to remove oil and film on the slides. The carriers will be sterilized by placing in evaporating dishes matted with two pieces of filter paper, heating them in a hot air oven for two hours at 180C, cooling and storing them at room temperature until use.

Using a positive displacement pipet, a 0.01 mL (10 µL) aliquot of each culture will be transferred onto a one-square inch area on the sterile carriers (in Petri dishes) and immediately spread uniformly over the entire area with a sterile glass rod. Each dish will be covered promptly and the operation will be repeated for the rest of the carriers. Carriers will be dried for 30-32 minutes at 36±1C. The humidity level of the incubator during the drying phase required for the inoculated carriers will be monitored and reported. Inoculated carriers will be used for testing within two hours of drying.

C. Test substance preparation:

Each lot of the substance will be used as supplied by the sponsor in individual packages (one wipe per packet).

Special Instructions before use include:

1) *Each packet will be numbered and will include the sponsor-measured weight value.*
2) *Each packet will be weighed by Microbac before testing to verify that the weight matches the sponsor measure weight within +/- 2% range. If it does not, the weight will be documented and the wipe will not be used.*
3) *The packaging for the test towelette* (wipe) *will be massaged to distribute the liquid test agent into the towelette matrix before opening.*
4) *The exterior of each packet will be wiped using a fresh alcohol prep pad to help prevent external microbial contamination that might interfere with the study.*
5) *The towel will be removed from the packet and then used for the treatment of 10 carriers based on the procedures outlined in Section D (Test).*

D. Test:

Note: The temperature and humidity level of the laboratory during the test phase will be monitored and reported.

Sixty carriers per lot will be evaluated whereas a single wipe will be used to treat each set of 10 carriers based on the following:

Each towelette will be folded in such a way that one towelette will be used to treat ten carriers. Each carrier (maintained in the Petri dish that was used for carrier inoculation) will be wiped for the specified time requested by the sponsor. The area of the towelette used for wiping will be rotated to expose a new, unused surface for each carrier, allowing a maximum surface area of the towelette to be used over the course of the procedure.

Initially the towelette will be folded lengthwise twice and then folded five times inward beginning from the far end. Then the outside edges will be pulled upward to form a "U" shape and grasped on one side with the thumb and on the other side with the index and middle finger.

The initial contaminated carrier (for each 10 replicate set) will be wiped using two complete horizontal strokes, with one right to left and back to right considered as one stroke; and then wiped using two complete vertical strokes, with one up to down and back to up considered one stroke for a total of four complete strokes.

After the treatment of the initial carrier for the 10 replicate set, the area of the towelette used for wiping will be rotated to expose a new, unused surface for each carrier while allowing a maximum surface area of the towelette to be used over the course of the procedure.

The used end will be flipped upward towards itself, reoriented appropriately and then used to wipe the next carrier. The next three carriers will be wiped in a similar fashion whereas the used portion will be folded up-and-over each time.

Once five carriers have been wiped, the second lengthwise fold will be unfolded and refolded in the opposite direction. The towelette will be refolded five times in the same manner as the original pre-folded state and the above procedure for wiping the first five carriers will be repeated for wiping the last five carriers. This process will be repeated until a total of ten carriers have been wiped with one wipe.

Once treated, each carrier will be held in a horizontal position for the exposure time as specified by the sponsor. After the contact period, each carrier will be transferred to tubes containing 20 mL of the Neutralizer using sterile forceps within the ±5 sec. (or ±3 sec.) time limit and shaken thoroughly. For products with ≤1 minute contact time, the transfer will be made within ±3 seconds. The slide can touch both the interior sides of the Petri dish and the neutralizer tube during the transfer, but care will be taken to avoid this contact as much as possible.

All subculture tubes containing the carriers will be incubated for 48±2 hours at 36±1C. All observations will be recorded as growth or no growth.

E. Controls:

1. Sterility controls:

   One sterile carrier will be added to a tube of Neutralizer and incubated with the test to demonstrate the sterility of the media used in the study.

2. Viability controls:

   Two inoculated carriers will be independently transferred into tubes Neutralizer and incubated with the test to serve as comparison for the test cultures.

3. Neutralizer effectiveness:

   Using six sterile carriers per lot, the test substance will be applied to the carriers, held for the contact time and subcultured into individual tubes of Neutralizer based on the procedures used for the test (with the exception that two replicates, per test substance will be treated).

   To each tube, fewer than 100 colony forming units (CFU) of the challenge microorganism will be added and the count of the bacteria inoculated into these tubes will be confirmed in duplicate TSA plates. The tubes and plates will be incubated with the test.

4. Carrier counts:

   For each group of 60 test replicates, per lot, triplicate replicates will be processed immediately before and after processing the test for a total of six triplicate sets.

   Dried inoculated carriers will be placed individually into tubes containing 20 mL LB. The tubes will be immediately vortexed for 120±5 seconds. After vortexing, serial ten-fold dilutions of each suspension will be performed in PBDW blanks. Duplicate one mL aliquots from selected dilutions will be plated in TSA pour plates. Diluting and plating will be completed within 2 hours after vortexing. All plates will be incubated with the test and the average CFU/carrier determined.

5. Bacteriostasis control:

   If, after two days incubation, no growth is observed in any of the test tubes, at least 20% of the test tubes will be streaked onto suitable agar media and incubated for 24±2 hours at 36±1C. No growth on these plates will negate bacteriostasis as the cause for lack of growth in the test tubes.

6. Confirmation of challenge microorganism:

   All viability controls and at least 20% of the test tubes showing growth will be streaked onto TSA plates. The same tubes will also be streaked on MSA. All plates will be incubated for 24±2 hours at 36±1C. Gram stains will be performed from these streaks to confirm growth of the challenge microorganism.

TEST ACCEPTANCE CRITERIA:

The test will be acceptable for evaluation of the test results if the criteria listed below are satisfied. The study director may consider other causes that may affect test reliability and acceptance.

- Per the AOAC Germicidal Spray Products Test, for, *Staphylococcus aureus*, the average of the carrier counts must be between 1.0×10^5 and 3.2×10^6 CFU/carrier. Average results that do not meet this criterion will be interpreted based on the following criteria:
  - For average counts that are below the stipulated range, the test must be repeated if the performance standard is achieved
  - For average counts that are below the stipulated range, the test does not need to be repeated if the performance standard is not achieved
  - For average counts that are above the stipulated range, the test does not need to be repeated if the performance standard is achieved
  - For average counts that are above the stipulated range, the test must be repeated if the performance standard is not achieved
- The $\log_{10}$ density (LD) for each carrier will be determined based on the following:
  - Dilutions yielding counts up to 300 CFU will be used.
  - Plate counts of 0 will be included in the calculations.
  - The CFU/mL (of broth) will be calculated:

$$\text{CFU/mL} = \frac{(\text{avg. CFU for } 10^{-x}) + (\text{avg. CFU for } 10^{-y}) + (\text{avg. CFU for } 10^{-z})}{10^{-x} + 10^{-y} + 10^{-z}}$$

  - The CFU/carrier will be calculated by multiplying the CFU/mL by the volume of broth into which the bacteria were harvested from the carrier by vortex-mixing (20 mL).
  - The LD for each carrier will be calculated by taking the Log_{10} of the density (per carrier).
- The recovery broth with neutralizers must be proven effective
- The sterility control must be negative for growth
- The viability control must be positive for growth
- The neutralization confirmation tubes must show growth following inoculation with <100 CFU per tube to confirm effective neutralization.
- The purity of the challenge microorganism must be confirmed based on the procedures employed for confirmation

PRODUCT EVALUATION CRITERIA:

According to the EPA, the test substance passes the test if visible growth is observed in no more than one subculture broth tube (≤ 1/60) per lot and the controls meet their stipulated criteria. There is no statistical method proposed for this protocol.

DATA PRESENTATION:

The final report will include the following information:

- The number of positive carriers per test substance (or lot).
- The average colony-forming units per carrier.
- The results of all controls.

PERSONNEL AND TESTING FACILITIES:

A study director will be assigned before initiation of the test. Resumes for technical personnel are maintained and are available on request. This study will be conducted at Microbac, 105 Carpenter Drive, Sterling, VA 20164.

CONFIDENTIALITY:

All data generated at Microbac are held in strictest confidence and are available only to the sponsor and the sponsor designated authorities (if applicable). In turn, no reference to Microbac's promotion of the evaluated test articles may be made public by the sponsor.

REPORT FORMAT:

The report will contain all items required by 40 FR Part 160.185 and EPA 810.2200 and be in compliance with EPA PR Notice 2011-3. Microbac employs a standard report format for each test design. Each final report will provide at least the following information:

- Sponsor identification
- Test substance identification
- Type of assay and project number
- Study start and end time (clock time)
- Interpretation of results and conclusions
- Test results presented in tabular form
- Methods and evaluation criteria, if applicable
- Dates of study initiation and completion (GLP studies only)
- Signed Quality Assurance and Compliance Statements (GLP studies only)
- Certificate of Analysis (provided by the sponsor)

REGULATORY COMPLIANCE AND QUALITY ASSURANCE (GLP studies only):

This study will be performed in compliance with the US Environmental Protection Agency's Good Laboratory Practices (GLP) regulations, 40 CFR 160 (note: information on the identity, strength, purity, stability, uniformity, and dose solution analysis of the test substance resides with the sponsor of the study unless otherwise stated).

The Quality Assurance Unit of Microbac will inspect the conduct of the study for GLP compliance. The dates of the inspections and the dates that findings are reported to the study management and study director will be included in the final report.

PROTOCOL AMENDMENTS AND DEVIATIONS:

Any protocol amendment(s) and protocol deviation(s) identified will be reported in project sheet(s) and included in the final report.

RECORDS TO BE MAINTAINED:

For all GLP studies, the original signed final report will be sent to the Sponsor. A draft report will be provided to Sponsor for review prior to finalization of the report. All raw data, protocol, protocol modifications, test substance records, copy of final report, and correspondence between MICROBAC and the sponsor will be stored in the archives at MICROBAC, 105 Carpenter Drive, Sterling, Virginia 20164 or in a controlled facility off site. All changes or revisions to this approved protocol will be documented, signed by the study director, dated and maintained with this protocol. The sponsor will be notified of any change, resolution, and impact on the study as soon as practical.

The proposed experimental start and termination dates; additional information about the test substance; challenge microorganism used and the type of neutralizers employed in the test will be addressed in a project sheet issued separately for each study. The date the study director signs the protocol will be the initiation date. All project sheets issued will be forwarded to the study sponsor for appropriate action.

MISCELLANEOUS INFORMATION: The following information is to be completed by sponsor before initiation of study:

A. Name and address: Professional Disposables International, Inc.
Two Nicepak Park
Orangeburg, NY 10962

B. Test substance information:

Test substance name			Active Ingredient(s)
BACKSPIN NO-RINSE FCSS (EPA REG. No. 9480-13)			Lot No 1 = 0.0365% QUAT Lot No 2 = 0.0367% QUAT Lot No 3 = 0.0366% QUAT
Lot No. 1	Lot No. 2	Lot No. 3	
PDI-0025-128A	PDI-0025-0129A	PDI-0025-0130A	

All lots manufactured on 11/2/18, 11/8/18, 11/8/18 Expiration date: 11/2/19, 11/8/19

C. Test Conditions:

Preparation of the Test substance	Not applicable, Ready to Use		
Organic load in the inoculum	☒ yes	☐ No	
Lower Certified Limit (LCL)	☒ yes	☐ No	☐ Not applicable
Contact time (one)	FIVE (5) MINUTES		
Contact temperature	Ambient Room Temperature (20±1C)		

D. Precautions/storage conditions: refer to MSDS or C of A: ☒ yes ☐ no

REPORT HANDLING AND STUDY CONDUCT: ☒ EPA ☒ Health Canada, GLP

PROTOCOL APPROVAL:

Sponsor Signature: Phyllis Vitolo Date: 11/15/18
Phyllis Vitolo, Clinical Microbiology Research Fellow /Regulatory Affairs
Professional Disposables International, Inc.

Study Director Signature: Hilary Kurland Date: 11/26/18
Hilary Kurland, Microbac